Membrane Traffic: Catching the Lysosome Express

Recent work shows that specific adaptor interactions may sort receptors into distinct cohorts of clathrin-coated vesicles. These different vesicle populations deliver cargo to sorting endosomes of differing motilities and maturation rates, thereby determining the probability of receptor recycling and the duration of endosomal signaling.

Michael J. Clague and Dean E. Hammond

Following internalisation in clathrin-coated vesicles (CCVs), epidermal growth factor receptors (EGFR) and transferrin receptors (TfR) are delivered to a tubulo-vesicular compartment that has been operationally defined as the early or sorting endosome [1]. Early endosomes have long been known to display directed movement that is microtubule dependent [2], overall centripetal and temporally coupled to their acquisition of late endosomal characteristics (a process termed maturation), such as the exchange of the small GTPase Rab5 for Rab7 and increased acidity [3–5]. TfR recycles to the plasma membrane through tubular elements of the early endosome, whereas the EGFR is sorted into lumenal vesicles of a maturing multivesicular body (MVB). Late endosomes/MVBs are then able to deliver material to lysosomes by direct fusion [6]. In a new study using live-cell fluorescence microscopic imaging and particle tracking, Lakadamyali *et al.* [4] have now sub-divided early endosomes into a static fraction and a rapidly maturing, motile fraction. Remarkably they find that CCVs bearing cargo destined for lysosomal degradation are 'fast-tracked' into this pathway by selective delivery to the motile fraction, whereas the recycling Tf molecule is delivered to both the static and motile compartments. This finding together with other recent studies [7,8] suggest that not all plasma-membrane-derived CCVs are created equal; some degree of cargo sorting into vesicles that contain distinct factors governing their route must be occurring.

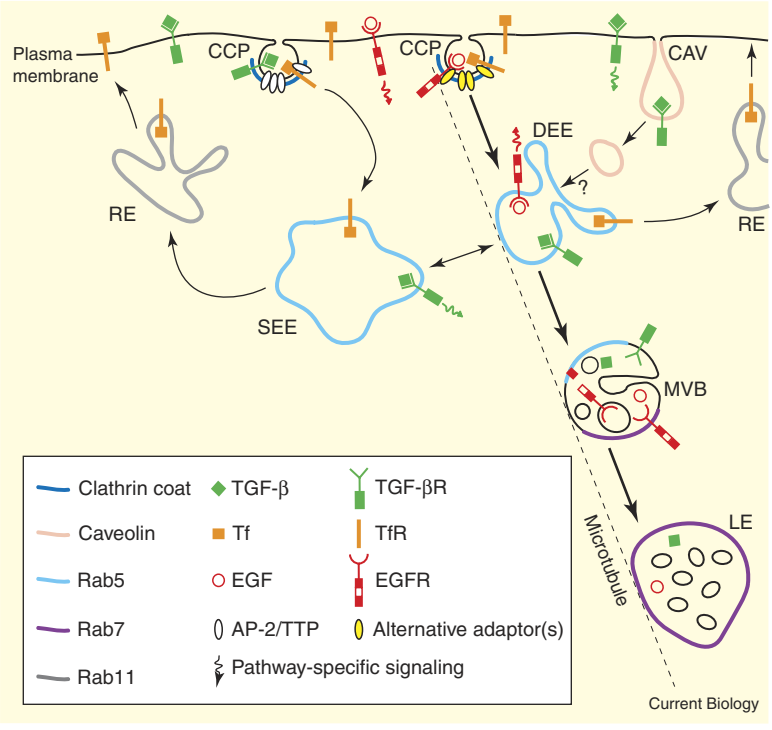

Figure 1. Specific entry portals influence receptor fate and signaling outputs.

Receptors can be internalised from the plasma membrane through different types of clathrin-coated vesicles budding from clathrin-coated pits (CCP) by linkage to specific adaptor proteins. These adaptors either directly connect with rapidly maturing, dynamic early endosomes (DEE) along microtubule tracks or deliver to a more abundant static early endosome (SEE) population. Caveolae offer another internalisation pathway utilised by TGF-βR to target receptors for degradation. Route selection to different endosomal populations is proposed to determine signal output and duration. RE, recycling endosome; LE, late endosome; MVB, multivesicular body.

The complement of Rab small GTPases has been used to provide a molecular signature for the identity of subcellular compartments [9]. Rab5 is an established marker for early endosomes, but new tools are now allowing this population to be dissected into further components characterised by coincident markers or physical properties. In the recent work Lakadamyali *et al.* [4] show that, in BS-C-1 cells, about 35% of Rab5-positive endosomes show directed movement, whilst the remainder are relatively static over a 100 second time window [4]. Occasionally transitions from static to motile endosome are observed immediately preceding the acquisition of Rab7. For both internalised EGF and low density lipoprotein (LDL), which are destined for lysosomes, around 80% is delivered directly to fast, maturing endosomes, even though the LDL receptor itself will be recycled. In contrast, vesicles carrying Tf are non-discriminately delivered to both sets of Rab5-positive endosomes (from which it can recycle). Thus most Tf molecules are delivered to the larger static population (65%). When microtubules are disrupted through nocodazole treatment, the selective targeting of LDL particles is lost. A possible model to explain this observation is that normally one class of CCVs (containing LDL or EGF) are themselves transported along microtubules until they

encounter a cognate endosome, whereas the majority (containing Tf) take a much less efficient random walk (Figure 1). Some potential CCV adaptor proteins (such as epsin, AP180 and HIP1) that link receptors to the clathrin lattice contain E/ANTH domains, which can bind directly to tubulin [10]; it is possible that other adaptors might bind to specific motors.

Another recent paper has used similar methods to track endosomes labelled with fluorescent Rab5 over longer time periods in A431 cells. Rink *et al.* [3] observe that the Rab5 content of endosomes can fluctuate due to 'homotypic' fusion events between Rab5-containing endosomes and subsequent loss of excess Rab5. Effectively this can lead to the coalescence of non-recycling cargo (such as LDL) into fewer and bigger structures. These events are much more common than maturation events associated with exchange of Rab5 for Rab7 and may in fact serve to maintain the stability of Rab5 endosomes, which have a half-life of about 15 minutes. The Rab5–Rab7 exchange is insensitive to nocodazole treatment indicating that some aspects of endosome maturation are microtubule independent.

How may pre-endosomal sorting occur? Some receptors use alternative internalisation pathways, such as caveolar uptake [11]. In fact, receptors such as TGF-βR and EGFR may utilise both CCV-mediated and caveolar pathways [12,13]. Although route selection by the EGFR can depend on the stimulus dose and ubiquitination status of the receptor [12], Lakadamyali *et al.* [4] have used experimental conditions under which all the internalisation events appear to emanate from clathrin-coated pits. The answer therefore seems instead to lie with the different adaptor molecules that link receptors to the clathrin lattice [14]. Tf, EGF and LDL uptake all occur via saturable, but non-competitive pathways [15]. RNA-interference-mediated knockdown of the best characterised and most abundant adaptor protein, AP-2, inhibits uptake of Tf but not that of LDL and EGF [4,16,17].

Furthermore, the density of slowly maturing static Rab5 endosomes is reduced eightfold in these cells [4] and a higher proportion of residual endosomes are now motile. Only about 15% of fluorescent-clathrin-coated pits colocalise with LDL, whereas Tf is associated with more than 90% of such pits. The LDL findings may be attributable to the use of specific adaptor proteins such as Disabled-2, which, when present in a clathrin-coated pit, doubles the probability of LDL association [4], and ARH, an adaptor protein required for LDL internalisation in some tissues [18]. Another adaptor molecule, TTP, has also recently been shown to play a specific role in Tf uptake [7].

The notion that receptors may be pre-sorted at the plasma membrane for delivery to distinct sorting endosomes has previously been suggested [13]. Accordingly, inhibition of either the clathrin-mediated or caveolar pathway had opposite effects upon TGF-β signalling: if clathrin-mediated uptake is blocked, then receptor degradation is promoted, whereas if caveolar uptake is inhibited, then the receptor lifetime is enhanced and Smad2 activation is increased. The new work by Lakadamyali *et al.* [4] is important because it establishes that sorting at the plasma membrane can be much more subtle than previously thought: receptor fate and signaling can be dictated by entry into sub-classes of CCV that deliver to distinct endosomes, presenting specific effectors or sequestering the receptor into lumenal vesicles at different rates. One final new consideration is that signaling receptors are not mere passengers, but can actively promote compartmental remodelling. Recent data have suggested that internalisation of activated EGFRs increases the number of MVBs and also the number of lumenal vesicles they contain [19]. So, EGFR not only catches the lysosome express, but it may drive it too!

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Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, UK.
E-mail: clague@liv.ac.uk

DOI: 10.1016/j.cub.2006.05.009

Myelin Biogenesis: Sorting out Protein Trafficking

Myelin biogenesis is a complex process involving coordinated exocytosis, endocytosis, mRNA transport and cytoskeletal dynamics. Recent studies indicate that soluble neuronal signals may control the surface expression of proteolipid protein, a process that involves reduced endocytosis and/or increased transport carrier recruitment from an intracellular pool.

Mihaela Anitei and Steven E. Pfeiffer

Myelin is a dynamic, multilamellar membrane which ensheathes axons, providing the structural basis for saltatory nerve conduction which allows for increases in the speed of action potential propagation and dramatic savings in both energy consumption and space requirements; without myelin, neurons would have to have significantly larger diameters to achieve the same conduction speed [1–3]. The myelin sheath also participates in bidirectional communication with both its partner axons and the environment [1–3]. For example, myelin regulates axon diameter and is a key player in ion channel clustering at nodes of Ranvier [4]. Myelin-associated glycoprotein can inhibit neurite outgrowth during axonal regeneration; reciprocally, neurons regulate myelin gene expression, oligodendrocyte survival [1] and, as recently reported by Trajkovic *et al.* [5], myelin proteolipid protein recruitment to the membrane. Loss or damage of myelin results in serious neurological disorders such as multiple sclerosis [1]. Remyelination is limited to a few lamellae, restoration of function is generally poor, and therapies remain suboptimal. The interdependence of myelin and axons takes on increasing importance with recognition of axonal degeneration in demyelinating disease.

Myelin biogenesis is a major part of brain development. As oligodendrocytes enter terminal differentiation, coordinated myelin gene expression is initiated, oligodendrocyte processes interact with axons, and myelin is produced as a specialization of the oligodendrocyte plasma membranes on a remarkable scale of approximately 5–50 x 10^3 μm^2 membrane per cell per day [2,3]. Although myelin-like membranes are synthesized in culture without neuronal contact, *in vivo* the quantity and stability of myelin is strongly enhanced by oligodendrocyte–neuron interactions [2,3]. While myelin has a relatively simple pattern of major proteins, there are myriad quantitatively, though certainly not functionally, 'minor' proteins [6], some of which have been implicated in demyelinating diseases [7]. Further, myelin has multiple domains: myelin basic protein and proteolipid protein are found abundantly in compact internodal myelin; oligodendrocyte specific protein is localized to junctions that spiral through the myelin sheath; neurofascin-155 is concentrated at paranodes; and myelin-associated glycoprotein and myelin oligodendrocyte protein are concentrated in periaxonal and outer lamellae, respectively [2,4].

This asymmetric distribution of proteins provides myelin with the potential for functional diversity and compartmentalization of activity. However, with the long distances myelin membrane components may need to travel along oligodendrocyte processes to reach their target membranes, this also imposes additional burdens on the biosynthetic and trafficking mechanisms. One would therefore expect that molecules that regulate and coordinate the trafficking and recruitment of transport carriers to the plasma membrane and cytoskeletal dynamics would be essential for oligodendrocyte differentiation and myelin biogenesis. For example, mRNA molecules encoding myelin basic protein are transported to compact myelin in granules that contain specific components of the translation and transport machineries [2]. Exocytic transport regulators, such as Rab3a, the exocyst components Sec8 and Sec6, and the exocyst regulator RalA, are expressed at high levels in myelin; Rab3a and the v-SNARE synaptobrevin-2 are up-regulated during maturation of oligodendrocytes; and Sec8 is central for oligodendrocyte process growth and arborization [2,8,9]. A recent genetic screen in zebrafish [10] showed that N-ethylmaleimide sensitive factor, a protein critical for membrane fusion, is required for correct expression of myelin basic protein and formation of nodes of Ranvier.

Current hypotheses suggest that recycling endosomes play central roles in protein sorting and trafficking, both during plasma membrane recycling and as an intermediate step during cargo transport from the trans-Golgi network to the plasma membrane [11] (Figure 1). Trajkovic *et al.* [5] investigated how axonal signals might control myelin biogenesis,